PREPARATION AND BIOLOGIC ASSAY OF ESTERS OF 
\(\alpha\)-TOCOPHERYLHYDROQUINONE* 

BY MILTON FARBER, JULIA B. MACKENZIE, HARRIS ROSENKRANTZ, 
AND ADE T. MILHORAT 

(From the Departments of Psychiatry and Medicine, Cornell University Medical 
College, the Russell Sage Institute of Pathology, and The New 
York Hospital, New York, New York) 

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Both the oxidation product of \(\alpha\)-tocopherol, \(\alpha\)-tocopherylquinone, and 
the reduction product of the latter, \(\alpha\)-tocopherylhydroquinone (I), have 
been shown to possess vitamin E activity in preventing or curing nutritio- 
tional muscular dystrophy in the rabbit (1). While the quinone is far 
more stable than the hydroquinone (I), its antidystrophic activity is of a 
lower order. Since I, however, is extremely susceptible to atmospheric 
oxidation and since both compounds are soluble only in fats or fat sol- 
vents, which for the most part are not suitable for physiological use, our 
attention was directed to the synthesis of esters of I which might obviate 
these two disadvantages and still retain the biologic activity of the parent 
compound. The present communication is a report on the preparation of 
four esters of \(\alpha\)-tocopherylhydroquinone, the tetrasodium diphosphate 
ester (II), the disodium disuccinate (III), the diacetate (IV), and the 
triacetate (V), and the response of the dystrophic rabbit to the intrave- 
nous and oral administration of these substances. 

Preparation of Materials 

Tetrasodium \(\alpha\)-Tocopherylhydroquinone Diphosphate (II) — 10 gm. of 
\(\alpha\)-tocopherylquinone (0.022 mole), prepared by ferric chloride oxidation 
of \(dl\)-\(\alpha\)-tocopherol (1) and purified according to the method of Tishler 
and Wendler (2), were converted to the hydroquinone by means of sodium 
hydrsulfite reduction and dissolved in 50 ml. of dry pyridine. The solu- 
tion was cooled in ice and added to an ice-cold solution of 10 gm. of phos- 
phorus oxychloride (0.065 mole) in 50 ml. of dry pyridine. After standing 
4 hours, the reaction mixture was filtered and the residue washed with dry 

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dunds which made the present investigation possible. 

1 The water emulsion of I prepared according to Rosenkrantz and Milhorat (5) is 
suitable for parenteral use; however, the stability of the emulsion is limited. 

2 We are indebted to Merck and Company, Inc., Rahway, New Jersey, for the 
generous supply of \(dl\)-\(\alpha\)-tocopherol used in the preparation of I.
ether and discarded. The solvent was removed in vacuo and the pink residue extracted with two 100 ml. portions of ether. The ether solution was then extracted with five 50 ml. portions of 5 per cent sodium hydroxide solution, and the alkaline extract acidified with 10 per cent hydrochloric acid to liberate the diphosphate ester of \( \alpha \)-tocopherylhydroquinone, which was extracted with three 100 ml. portions of ether. After drying with anhydrous magnesium sulfate, the ether was removed and the residue dried in vacuo, leaving the ester as a light brown glassy solid.

The ester was dissolved in a minimal amount of water (with the aid of small amounts of 10 per cent sodium hydroxide solution, if necessary) and made just alkaline with 10 per cent sodium hydroxide solution. 2 volumes of ethanol were added and, after the solution had stood 24 hours, the supernatant solution was decanted, leaving the crude tetrasodium \( \alpha \)-tocopherylhydroquinone diphosphate (II) as a tan viscous oil, which solidified when it was triturated with acetone and allowed to stand 24 hours.

\[
\begin{align*}
I, R_1 = R_2 = H; \\
II, R_1 = PO_3Na_2, R_2 = H; \\
III, R_1 = COCH_2CH_2COONa, R_2 = H; \\
IV, R_1 = AC, R_2 = H; \\
V, R_1 = I = AC.
\end{align*}
\]

acetone was decanted after centrifugation, and the trituration process was repeated with fresh acetone. The nearly white, amorphous sodium salt thus obtained (4 to 5 gm., 27 to 32 per cent based on the starting quinone) was finally centrifuged and dried in vacuo.

Purification was effected by dissolving the salt in 60 ml. of warm methanol, centrifuging, and decanting the methanol solution from the small amount of insoluble material. To reprecipitate the salt, 120 ml. of acetone were added and the mixture warmed to 50°. After centrifuging, the supernatant solution was decanted, leaving the crude tetrasodium \( \alpha \)-tocopherylhydroquinone diphosphate (II) as a tan viscous oil, which solidified when it was triturated with acetone and allowed to stand 24 hours.

For analysis, the salt was reprecipitated as above until a constant pH value was attained. The pH of a solution containing 0.8 mg. per ml. was 8.0 (Coleman pH meter).

\[
\text{Analysis}^{*} - C_{32}H_{60}O_8P_2Na_4
\]

Calculated. C 50.00, H 7.23, P 8.89

Found. C 49.98, 49.86, H 6.84, 6.79, P 8.98, 8.84

*Microanalyses by Dr. Carl Tiedeke, Teaneck, New Jersey.
Disodium \(\alpha\)-Tocopherylhydroquinone Disuccinate (III)—A mixture of 25 gm. of succinic anhydride (recrystallized from benzene), \(\alpha\)-tocopherylhydroquinone (from the reduction of 10 gm. of purified quinone), and 50 ml. of dry pyridine was heated under nitrogen at 65-70° for 4 hours. The reaction mixture was cooled, centrifuged, the supernatant solution decanted, and the residue washed with two 25 ml. portions of ether. The combined supernatant solutions were evaporated in vacuo, and the residue shaken for 10 minutes with 200 ml. of 1 per cent hydrochloric acid. After filtration and washing with 1 per cent hydrochloric acid, the residue was suspended in 150 ml. of water and 10 per cent sodium hydroxide solution was added in small amounts with vigorous shaking until solution was complete. The alkaline solution was then extracted with three 100 ml. portions of ether, small amounts of ethanol being added after shaking to facilitate separation of the resultant emulsion into two layers. After the aqueous layer was acidified and boiled to remove ether, the crude disuccinate ester was isolated as a gummy yellow solid, which was dissolved in ethanol, cooled to 0°, and made just alkaline (pH 8, hydrion paper) with ethanolic 1 N sodium hydroxide solution. After removal of the solvent under reduced pressure, treatment of the gummy residue with acetone as in the previous preparation yielded 3 to 4 gm. (20 to 26 per cent) of powdery, nearly white disodium \(\alpha\)-tocopherylhydroquinone disuccinate (III), similar in properties to the phosphate salt. The pH of a solution containing 0.8 mg. per ml. was 7.7.

For purification, the salt was reconverted to the free acid ester (m.p.\(^4\) 144.5–149°) by acidification, and an ethanol solution of the free acid ester was made just alkaline to phenolphthalein with 1 N ethanolic sodium hydroxide solution. About 5 per cent excess free acid ester was then added to insure the absence of excess sodium hydroxide, and the solid sodium salt isolated as before. This procedure was repeated to yield the analytical sample.

Analysis—\(C_{47}H_{68}O_{19}Na_{2}\)
Calculated. C 64.14, H 8.44, Na 6.64, acidification equivalent\(^6\) 346
Found. C 63.88, 63.98, H 8.20, 8.31, Na 6.82, 6.68, acidification equivalent 364, 365

\(\alpha\)-Tocopherylhydroquinone Diacetate (IV)—This material was prepared by the method of Tishler and Wendler (2), and after recrystallization from water-ethanol had a melting point of 64.5–65.5°, softening at 62°.

\(\alpha\)-Tocopherylhydroquinone Triacetate (V)—The method of John, Dietzel, and Emte (3) was employed, but it was necessary to recrystallize (with

\(^4\) All melting points are corrected.

\(^6\) By titration with standard hydrochloric acid to a methyl orange end-point.
Esters of α-tocopherylhydroquinone seeding) the material from ethanol six times to obtain crystals with a constant melting point (73.5-74.5°C).

Methods

The general procedures employed for the assay of vitamin E in the rabbit have been fully described in an earlier publication (1). II and III were given intravenously at a concentration of 2.5 mg. per ml. in either distilled water or isotonic saline. Concentrations higher than the above were less well tolerated. IV and V were administered intravenously in 10 per cent ethanol-propylene glycol and are less soluble than II or III, requiring slight warming of the solvent to effect solution. For oral assay, the esters were given by stomach tube to rabbits depleted of vitamin E, which had been fasted previously for 5 hours.

RESULTS AND DISCUSSION

The response of rabbits suffering with stage II dystrophy, as described by Mackenzie and McCollum (4), to the intravenous and oral administration of the four esters of α-tocopherylhydroquinone (I) is presented in Table I. It is clear that, of the compounds tested, the triacetate (V) alone seemed to be ineffective, while the other three, namely the tetrasodium diphosphate (II), the disodium disuccinate (III), and the diacetate (IV), possessed a comparable degree of vitamin E activity when assayed intravenously. Each of these esters was given in amounts calculated in terms of their equivalent to the parent hydroquinone (I), in order to facilitate comparison of potency. On this basis it is clear that the esters of I are less potent in vitamin E activity than is free I itself. As was the case in the assay of I (1), increasing the level of the tested esters apparently did not prolong the duration of response, suggesting early excretion or limited storage capacity for these derivatives. A strong local edema was observed, and an occasional rabbit would die immediately after the intravenous administration of 20 mg. of the tetrasodium diphosphate (II). This response suggests a toxic effect, conceivably due to the strong surface-active properties of the compound. When smaller doses were given intravenously, the rabbits survived without any ill effects. No untoward effects were observed after the injection of the other esters.

With the exception of the disodium disuccinate (III), none of the esters tested was capable of showing antidystrophic activity when given per os at high levels6 (Table I). Apparently these orally inactive esters are not hydrolyzed to I or its oxidation product, α-tocopherylquinone, in the gastrointestinal tract, since the latter compounds show vitamin E activity

6 A small and questionable activity was shown by the diacetate (IV) in this assay (Table I).
TABLE I
Effect of Intravenous and Oral Administration of Esters of α-Tocopherylhydroquinone on Creatine Excretion of Dystrophic Rabbits

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Supplement</th>
<th>Dose (in terms of hydroquinone content)</th>
<th>Average creatine excretion on day prior to supplement</th>
<th>Average maximal drop in creatine excretion*</th>
<th>Average duration of lowered creatine excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg.</td>
<td>mg.</td>
<td>per cent</td>
<td>days</td>
</tr>
<tr>
<td>Intravenous route</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Tetrasodium α-tocopherylhydroquinone diphosphate (II)</td>
<td>5†</td>
<td>101</td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Disodium α-tocopherylhydroquinone disuccinate (III)</td>
<td>15</td>
<td>128</td>
<td>55</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>α-Tocopherylhydroquinone diacetate (IV)</td>
<td>20</td>
<td>60</td>
<td>53</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>α-Tocopherylhydroquinone triacetate (V)</td>
<td>20</td>
<td>89</td>
<td>78</td>
<td>4</td>
</tr>
<tr>
<td>Oral route</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Tetrasodium α-tocopherylhydroquinone diphosphate (II)</td>
<td>50</td>
<td>104</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Disodium α-tocopherylhydroquinone disuccinate (III)</td>
<td>100</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>α-Tocopherylhydroquinone diacetate (IV)</td>
<td>200</td>
<td>85</td>
<td>59</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>α-Tocopherylhydroquinone triacetate (V)</td>
<td>100</td>
<td>98</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>α-Tocopherylhydroquinone (I)</td>
<td>100‡</td>
<td>98</td>
<td>75</td>
<td>7.5</td>
</tr>
</tbody>
</table>

* Maximal drop in creatine (mg.) = \( \frac{\text{Creatine (mg.) at time of supplement}}{100} \)

† The response of two rabbits to the intravenous administration of 5 mg. of II was atypical, although quite striking. Both animals were supplemented while their creatine output was still nearly normal, namely 15 and 16 mg. daily. Both animals, however, were losing weight rapidly, and the amount of food eaten was between 5 and 10 gm. daily. Muscular weakness was also evident in both animals. These symptoms taken together are those seen in a stage I dystrophy as described by Mackenzie and McCollum (4). Following supplementation, the creatine output of the first rabbit averaged 33 mg. for 8 days and that of the second, 23 mg. for 10 days. The gains in body weight were 295 and 313 gm., respectively. By the 4th day after supplementation, the food intake of both rabbits averaged over 65 gm. daily, and all signs of muscular incoordination were completely gone.

‡ Assay data for I taken from an earlier publication (1).
ESTERS OF \( \alpha \)-TOCOPHERYLHYDROQUINONE

when given orally (100 mg.). Probably these three esters either are rapidly excreted or are converted to an inactive form in the gut.

It seems, therefore, that of the esters examined, the activity, both intravenously and orally, the stability, and the water solubility of III render it, in spite of its diminished potency, a possible substitute for the free \( \alpha \)-tocopherylhydroquinone in further studies.

The apparent complete lack of activity of the triacetate ester (V) suggests the possibility that a free tertiary hydroxyl group is required for the vitamin E activity of \( \alpha \)-tocopherylhydroquinone compounds, and work in this direction is in progress.

SUMMARY

The tetrasodium diphosphate, disodium disuccinate, diacetate, and triacetate of \( \alpha \)-tocopherylhydroquinone have been prepared. These compounds are stable and of known purity. The four esters were tested both intravenously and orally in dystrophic rabbits to determine their vitamin E activity in the alleviation of nutritional muscular dystrophy. While the first three compounds showed potency when given intravenously, the triacetate administered in a like manner was totally without effect. Only the disodium disuccinate ester showed marked activity when given orally at a high dosage level. The vitamin E potency of the esters of the hydroquinone in relieving dystrophy is somewhat less than that obtained with the hydroquinone itself, as judged by the number of days of suppressed creatine excretion and relief of the dystrophic syndrome. The stability, purity, and ready solubility of these derivatives of \( \alpha \)-tocopherylhydroquinone make them ideal for parenteral administration. These desirable features, coupled with their biologic potency, make these esters suited for experimental work.

BIBLIOGRAPHY

PREPARATION AND BIOLOGIC ASSAY
OF ESTERS OF \( \alpha \)-TOCOPHYLHYDROQUINONE
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